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| RESEARCH ARTICLE

Drought-Induced Modulation of Bioactive Compounds in Rice: A Comparative Study of Tolerant and Susceptible Varieties

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| ABSTRACT
In this research, the correlation between bioactive compounds and drought stress was studied in 11 rice varieties. The rice varieties were divided into treatment and control groups, and the plants were subjected to different drought conditions, including 3, 5, and 7 days without irrigation. Among the rice varieties, K8 was the most tolerant, whereas K11 was the most susceptible to water deficit. The results showed that total phenolic content increased dramatically in K8 and K11 under a 7-day treatment (71.397 and 51.381 mg gallic acid equivalent (GAE)/g, respectively). K8 showed higher antioxidant activities (DPPH = 8.832 µg/mL, ABTS = 1161.8 µg/mL, and reducing power = 1168.2 µg/mL) after 7 days of no irrigation. Contrastingly, the IC50 values indicate that K11 showed lower antioxidant activity (DPPH=16.261 µg/mL, ABTS=1944.5 µg/mL, and reducing power=3721 µg/mL) for the same variable.

| KEYWORDS

Rice, Drought Tolerance and Susceptibility, Total phenolic and Flavonoid contents, Antioxidant activities.

| ARTICLE INFORMATION

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1. Introduction

Fifty percent of the world's daily calorie intake comes from rice, making it the most important cereal crop, while 90% of the total rice production of the world comes from Asia (Maclean, 1997, Awika, 2011). 25% of the world's rice production area is rain-fed and lowland, accounting for about 18% of the world's production (Castillo et al., 2006). Asia, Africa, and South American countries could produce 481.5 (Full name of MMT) rice from 160 million hectares, as reported by (IRRI, 2006). Among abiotic stresses, drought is the most severe, affecting one-third of the world's rice production (Chaves and Oliveira, 2004; Passioura, 2006; Hassan et al., 2013). Crop production and agriculture face hazards due to the increase in global temperatures (Smith and Olesen, 2010; Boonjing and Fukai, 1996). Generally, a blend of molecular breeding techniques and practices could improve rice quality (Khush, 2005). Rice plants are affected at a sensitive stage during water shortage, which can enormously reduce both the quality and quantity of production (Islam et al., 2011; O, Toole, and Moya, 1981). Rice has a weaker drought tolerance than other cereal crops with high essential water requirements (Noelle et al., 2018). The tolerance and susceptibility of rice could be measured by monitoring its physiological properties (Islam et al., 2011). When rice faces water deficit, it is usually difficult to implement its normal growth functions (O, Toole JC, 2004). Drought stress at the vegetative stage is one of the sensitive periods during which rice plants can decrease tiller number and have a high negative impact on yield (Boonjing and Fukai, 1996). Rice recovery capability depends on genotypic mutations under water deficit (Lilley and Fukai, 1994). The morphology, physiology, and phytochemicals are the most parts of rice that are severely affected by water stress during growth (Chen et al., 2011; Jaleel et al., 2009; Gill and Tuteja, 2010; Fang and Xiong, 2015; Trenberth, 2011). Plants secondary metabolites have inhibitory or stimulatory effects on the emergence of different crop plants. Among the secondary metabolites phenolic and flavonoids compounds as well as anthocyanins play crucial antioxidant roles and scavenge free radicals that increase oxidative stress to destroyed biological molecules to decrease diseases such as cancer and cardiovascular (Finocchiaro et al., 2010, Gunaratne et al, 2013, Naczk and Shahidi, 2006, Pedro et al, 2016, & Ti,Li, et al., 2014). Due to their inhibitory and scavenging roles, phenolic compounds are highly essential to the food,

pharmaceutical, and cosmetic industries (Tuyen et al., 2018). Rice leaves and straws have two phytoalexin compounds (Cartwright et al., 1981 & Hasegawa et al., 2010). The level of antioxidant activity increases as the plant encounters water stress, while antioxidants play a protective role against oxidative stress damage (Wei et al., 2015). Antioxidants are the only sources that can control reactive oxygen species (ROS) in the human body, which can damage biological molecules such as lipids, proteins, carbohydrates, and DNA (Zhao et al., 2005). A major factor contributing for reduced crop productivity is the accumulation of Reactive Oxygen Species (ROS) under various environmental stresses (Wei et al., 2015).

Hence, this study aims is to 1. Determine the drought tolerance and susceptibility of rice in the vegetative stage. 2. Evaluate Phytoalexins and their correlations in rice cultivated under drought and control conditions including total phenolic, total flavonoid, antioxidant activities. 3. Identify essential compounds from rice leaves under drought and control conditions.

2. Material and Methods

2.1 Plant Materials and Treatment

Eleven rice (*Oryza sativa* L.) cultivars were cultivated in the greenhouse. The seeds were sterilized and soaked in water at 45 ℃ and kept for 20 minutes, after which the seeds were immersed in 25 ℃ water and stored for two days. The seeds were washed with distilled water three times every day. The Seeds were pre-germinated in Petri dishes at room temperature for three days. Plastic plates (length: 50 cm, width: 30 cm) were filled with 7 cm of sterilized soil where seeds were sown at a depth of 1-2 cm in the greenhouse under optimal conditions (25-30 ^{0}C, night/day cycle, 14 hrs. photoperiod and 85% soil moisture).. 20-days seedlings were then transplanted to Wagner pots (30 cm high and 20 cm diameter). The plants were irrigated daily for eight weeks to maintain 85% of soil moisture; at the same time measuring the soil moisture content using moisture meter (SM150-HH2 (Delta-T Devices Ltd., Cambridge, UK). The plantlets were divided into two groups: test and control. Plantlets were subjected to different drought conditions: they were kept without water for 3, 5, and 7 days, respectively. Watering was conducted for 2 days after each stage of drought to initiate recovery.

Furthermore, leaf rolling, leaf drying, leaf withering, and leaf recovery were measured in the test group. We found that soil moisture content in the treatment groups decreased from 85% to 65%, 85% to 50%, and 85% to 35% after three, five, and seven days, respectively. Leaf samples were then stored at - 4℃ for further analysis.

Table 1. Rice cultivars and their codes

No	codes	Cultivars
1	K1	DT 84
2	K2	DT 84 x BT LV
3	K3	K 1 x BT LV
4	K4	K 2 x NH 8 x DT 84
5	K5	H lin
6	K6	H lin x BT LV (NH 8 x DT 84)
7	K7	H lin x BT LV
8	K8	NH 8
9	K9	cho dao x NH1
10	K10	cho dao x BT LV
11	K11	cho dao X BT LV (NH 8 x DT 84)

2.2 Drought Screening

Drought tolerance was evaluated following Standard Evaluation Scale (SES) recommended by the International Rice Research Institute (IRRI).

Table 2. Evaluation of drought tolerance rice.

Scales	Explanation
Leaf rolling.	
0	No symptoms (normal leaves)
1	Leaves starts folding (light V-shaped)
3	Leaves folding (deep V-shaped)
5	Leaves cupped fully (U-shaped)
7	Two leaf margins touching (O-shaped)
9	Leaves rolled tightly
Leaf drying	
0	No symptoms (normal leaves)
1	Slight leaf tip drying (extended to less than 1/4 length of leaves)
3	Tip drying extended to 1/4 length in 25% of all leaves.
5	Tip drying extended from 1/4 to 1/2 length in at most 50% of all leaves
7	Tip drying extended to 2/3 length or more in at most 70% of all leaves
9	All plants dryly died
Leaf withering	
1	Leaves had a natural green color (account for 95% all of the leaves)
5	The backside of all leaves transferred to yellow accounted for 70%
9	Leaves transferred to yellow color
Leaf recovery	
1	90%–100% of plants were recovered
3	70%–89% of plants were recovered
5	40%–69% of plants were recovered
7	20%–39% of plants were recovered
9	0%–19% of plants were recovered

2.3 Phenotypic Properties

Plant height, number of tillers per hill, leaf number, root length, , fresh root weight, and root dry weight were determined by. We used the oven to dry the samples at 45 °C for 72 hours after obtaining the plants' fresh weight.

2.4 Preparation of Extraction

Eight leaf samples were dried in the oven at 45^0C for 72 hours, after which they were ground into fine particles. One gram of each sample was mixed with 100 ml of methanol (100%) and kept at room temperature for 24 hrs. The samples were shaken

(shaker SM – 60N, Tokyo, Japan) for two days to mix well. All samples were filtered by a (90mm) filter paper. 100 ml of Hexane (100%) was applied to the samples in a separatory funnel for 10 minutes. The samples were kept for three hours to separate the fatty acids and lipids. This action was repeated twice to achieve a 100% separation. The generated solvent was evaporated at 40 ℃ using a rotary evaporator (SB-350-Eyela, Tokyo, Japan) and dissolved in methanol for further analysis.

2.5 Total Phenolic Content (TPC)

The total phenolic content was evaluated using the Folin-Ciocalteu (FC) method, as reported by (Andriana et al., 2019). The standard gallic acid was measured at concentrations of (5-25 µg/ mL) in separated wells. Considering a volume of 20 µL of the diluted sample (1.0 mg/mL) and then a volume of 100 µL of Na_2Co_3 (7.5% w/v distilled water) and 80 µL of Folin (10% v/v distilled water) was added to each well, in 96-wells microplate. The reaction was performed in 30 minutes at room temperature, and the absorbance was read at 765nm using microplate reader (Multiskan^{TM} Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan). The gallic acid equivalent (GAE) of the total phenolic content measured in mg/gram extract **and** was expressed in r-value (r^2 = 0.996).

2.6 Total Flavonoid Content (TFC)

The total flavonoid content was measured following the method reported by. In this method, the standard was identified by (5-25 µg/mL), 100 µL of samples mixed with 100 µL of aluminum chloride (2% w/v distilled water) was added in a 96- wells microplates, kept for 15 minutes at room temperature. The absorbance of the reaction was measured at 430 nm using microplate reader (Multiskan^{TM} Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan). The total flavonoid content expressed as mg quercetin equivalent (QE) per gram, r value (r2 = 0.999). The quercetin equivalent (QE) of the total flavonoid content measured in mg/gram extract was recorded in r -value (r2 = 0.999).

2.7 Antioxidant Activities

2.7.1 Radical Scavenging Activity (DPPH)

Radical scavenging activity was measured according to the method described by Elzaawely and Tawata (2012). 80µl of samples was pipetted in a microplate with 40µL of 0.5 mM DPPH and 80µL of 0.1 mM acetate buffer (PH 5.5), incubated at room temperature for 30 minutes in the dark, and measured at 517 nm using Multiskan^{TM} Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan. BHT standard was used as a positive control (0.01-0.05mg/mL). The formula below was used to compute the percentage of the radical scavenging activity (DPPH):

$$DPPH\ (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ denotes the absorbance of the control and $A_{sample,}$ indicates the absorbance of the sample. The value of the IC_{50} inhibitory concentration was recorded in ppm (parts per million) where lower values denote greater DPPH radical scavenger activities.

2.7.2 Evaluation of Reducing Power

Power reduction was estimated using the method reported by Ahmad et al. (2019). A 0.1 mL of sample was mixed with 2.5mL of potassium ferricyanide (1%), 2.5mL of (0.2M) phosphate buffer (PH 6.5) and was incubated for 30 minutes at 50 ℃, and 2.5mL of (10%) trichloroacetic acid was included to the mixture. The mixture was centrifuged at 4000 rpm for 10 minutes. 2.5 mL of the mixture was mixed with 2.5 mL of water and 0.5 mL of ferric chloride (0.1%). The absorbance was read at 700nm by utilizing microplate reader (Multiskan^{TM} Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan). BHT standards (10-50µg/ml) was used as a positive control. A lower IC50 value indicates greater antioxidant activity. The activity of reducing power was calculated using the formula below:

$$Reducing\ power\ (\%) = 100 - [(A_{control} - A_{sample})/A_{control}] \times 100$$

Here, $A_{Control}$ is the absorbance without sample, and A_{sample} is an absorbance with the sample.

2.7.3 Measurement of ABTS

ABTS was measured according to the method described by Phung et al. (2017). A 2.45 mM of potassium persulfate and seven mM of (3- ethylbenzothiazoline-6-sulfuric acid) ABTS solution in the same volume (v/v) was incubated in dark place at room temperature for 16 hours to generate a reaction that measures the ABTS activities. After that, methanol was added to obtain an absorbance of 0.70±0.05 at 734 nm. In summary, 0.120 mL of ABTS solution was pipetted into a 0.024 mL sample in a microplate.

The mixture was then kept in the dark room at room temperature for 30 minutes. The absorbance was run at 734 nm using microplate reader (Multiskan^{TM} Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan). 10-50ppm BHT standard was used as a positive control. Finally, the ABTS was evaluated by the equation below:

$$ABTS\ (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Wherever $A_{control}$ and A_{sample} represent the absorbance without and with samples respectively. Here, a higher number of IC_{50} is equated to the lower antioxidant activity, vice versa. The IC_{50} scavenging concentration was computed at 50% ABTS.

2.7.4 Gas Chromatography-Mass Spectrometry (GC-MS)

The chemical constituents of the sample were identified using Gas Chromatography-Mass Spectrometry (GC-MS) on a JMS-T100 GCV (JEOL Ltd., Tokyo, Japan). We injected 1 µL of the sample into the GC-MS. The column used in this experiment was DB-5MS, 30 meters in length with an internal diameter of 0.25 µm (Agilent Technologies, J&W Scientific Products, Folsom, CA, USA). We chose Helium as the carrier gas, with and split ratio of 5.0/1.0. The GC oven temperature is as follows: From the outset, the temperature was 500 °C without a hold time. The programmed rate was 10^0C/min, and the final temperature was 3000C with a 20-minute holding time. We fixed the injector temperature at 300 °C and the detector temperature at 3200 °C. The mass range scanned between 29-800 amu. The data peak methods and control of the GC-MS was conduct using the JEOL's GC-MS Mass Center System version 2.65a software (JEOL Ltd., Tokyo, Japan) (Andriana et al., 2019).

2.8 Statistical Analysis

The experiment was conducted based in a completely randomized design (CRD) with three replications. The data analysis method is a one-way ANOVA using Minitab 16.0 (Minitab Inc., State College, PA, USA). The results are shown as means ± standard deviation (SD) and are followed by Fisher's error rate, with a statistical significance level of p=0.05.

3. Results

3.1 Effect of Drought Stress on Rice Leaves

The drought tolerance and susceptibility of eleven rice cultivars were evaluated using indicators such as leaf rolling, leaf drying, leaf withering, and leaf recovery. As shown in table 2, the following scores are used to measure the level of tolerance in rice varieties: 1-3: strongly tolerant; 3-5: medium tolerant; 5-7: weakly tolerant; and 7-9: susceptible. The results show that K1, K2, K6, and K10 are weakly tolerant. On the other hand, K11 was found to be susceptible to drought, whereas K8 was found to be drought-resistant in three categories: leaf rolling, leaf drying, and leaf withering. The remaining five cultivars were observed to have a medium level of tolerance. The results show that most cultivars have substantial leaf recoveries, ranging from 1.0 to 3.667. Meanwhile, K8 exhibits higher leaf recovery rate, while K9, K10, and K11 had the lowest levels of leaf recoveries. Considering the responses of the cultivars to water deficit on the indicators mentioned above, K11 is highly drought-susceptible, while K8 is the most tolerant variety in the study.

Table 3. Tolerance and susceptible levels under water deficit

No	Rice variety	Leaf rolling	Leaf drying	Leaf withering	Leaf recovering
1	K1	4.333±0.667	4.333±0.667	3.67±1.33	3.667±0.667
2	K2	4.333±0.667	3.667±0.667	3.67±1.33	3.667±0.667
3	K3	3.667±0.667	3.667±0.667	3.67±1.33	3.667±0.667
4	K4	3.667±0.667	4.333±0.667	3.67±1.33	3±0
5	K5	3.667±0.667	4.333±0.667	2.33±1.33	3.667±0.667
6	K6	4.333±0.667	4.333±0.667	3.67±1.33	1.667±0.667
7	K7	3.667±0.667	4.333±0.667	5±0	1.667±0.667
8	K8	2.333±0.667	1.667±0.667	2.33±1.33	1±0
9	K9	3±0	3±0	3.67±1.33	4.333±0.667
10	K10	4.333±0.667	3.667±0.667	5±0	4.333±0.667
11	K11	5±0	5±0	5±0	4.333±0.667

Value means ± standard deviation (SD) (n=3). Grades of drought tolerance: (1) leaf rolling: 0=normal leaves, 1=light V=shaped leaves, 3=deep V=shaped leaves, 5=U-shaped leaves, 7=O-shaped leaves, 9=tight rolled leaves, (2) leaf drying: 0-normal leaves, 1-top of leaves are dried lightly, 3-leaves are dried up to 1/4 of leaf length, 5=1/4=1/2 of leaves are dried, 7=more 2/3 of leaves are dried, 9=leaves entirely dead, (3) leaf withering: 1=leaves are naturally green, 5=backside of leaves transfer to yellow color, 9=leaves totally transfer to yellow color, (4) recovering: 1=plants are covered from 90% to 100%, 3=plants are covered from 70% to 89%, 5=plants are covered from 40% to 69%, 7=plants are covered from 20% to 39%, 9=plants are covered from 0% to 19%.

3.2 Effect of Water Deficit Stress on the Phenotypic Traits of Rice

The phenotypic traits were examined using parameters such as plant height (cm), tiller number per hill, leaf number, root length (cm), root fresh weight (g) and dry root weight (g). As indicated in table 4, the plant heights ranged from 77.33 to 115.67cm, while the tiller number per hill spans from 10 to 26 cm. The Leaf numbers fluctuated between 60 to 119, and the weight of the fresh and dry roots was between 37.33 to 117.39 g, and 7.423 to 20.33 g respectively. Meanwhile, K8 and K11 reveal the lowest and highest numbers of plant heights and tillers per hill. This indicates that K8 is resistant to drought, whereas K11 is drought-susceptible. Intuitively, a higher plant height and tiller count indicate a need for increased water consumption. In the same vein, there was a lower number of leaves for K8 and a higher number of leaves for K11, indicating a high level of tolerance in K8. The data follow the same pattern for fresh and dry root weight, with K8 and K11 showing lower and higher weight, respectively. Table 4, therefore, shows that tiller number per hill, leaf number, fresh root weight, and root drought weight play a cardinal role in determining the level susceptibility and tolerance of the rice cultivars.

Table 4. Phenotypic characterization under drought stress

Variety	Treatment	Plant height (cm)	Tiller number per hill	Leaf number	Root length (cm)	Root fresh weight (g)	Root drought weight (g)
K1	Control	97±2.65a	18.333±1.528a	91.67±7.64a	30.33±2.52a	70.05±6.21a	12.367±1.419a
	W3	94±1.73ab	18.333±1.528a	94±7.81a	30.33±2.31a	69.66±3.89a	12.07±1.512a

	W5	91.67±2.08ab	18.667±1.528a	91.67± 5.86a	30±1.73a	68.36±2.58 a	11.9±1.323a
	W7	89±4.36b	18.667±1.528a	93.33± 7.64a	29.67±2. 08a	68.51±4.06 a	11.6±1.52a
K2	Control	115.67±5.36a	19 ±5.2a	101.7± 31.8a	31.33±2. 08a	96.16±13.4 2a	14.8±3.7a
	W3	105.33±3.48a	18.33±2.89a	107.33 ±6.43a	31±1.73a	97.2±5.1a	15.18±1.509a
	W5	101±5.29a	18.667±0.577a	101.67 ±9.07a	30.667±1 .528a	93.67±7.88 a	15.73±1.456a
	W7	100.33±7.17a	18.67±8.96a	91.7±4 1.9a	29.67±2. 52a	85.33±14.5 4a	18.43±7.85a
K3	Control	115±3a	14±1a	71.67± 7.64a	35.33±3. 06a	82.49±7.06 a	12.25±2.26a
	W3	105.33±3.06ab	14±1a	71±7.5 5a	33.667±1 .155a	68.77±5.11 ab	11.42±2.41a
	W5	96±6.08b	13.333±1.155a	69±6.5 6a	34±1a	60.66±6.79 b	9.5±1.86a
	W7	93.67±7.77b	13±1a	69±6.2 4a	31±3.46a	53.57±9.4b	7.82±2.27a
K4	Control	81.67±7.57a	14.67±2.52a	78±14. 73a	29±3.61a	62.9±25.6a	9.33±5.1a
	W3	80±4.58a	13.33±2.89a	74±12. 49a	29±3.61a	61.2±22.2a	9.85±4.11a
	W5	77.33±4.04a	12±3.46a	66±10. 15a	28.67±3. 79a	58.7±14.03 a	10.32±3.22a
	W7	80.33±10.02a	10.33±4.62a	63±14. 8a	28.67±3. 79a	59.37±7.57 a	11.64±2.41a
K5	Control	99±8.19a	22±5.57a	106.3± 24.8a	30.67±2. 08a	71.6±25.3a	14.34±5.31a
	W3	95.67±6.03a	23±3a	114±1 5.1a	32±3.61a	63.02±6.8a	13.173±1.357a
	W5	89.33±4.51a	25±5a	113.33 ±16.07 a	32.33±5. 51a	59.08±6.62 a	13.137±1.275a
	W7	83±13a	24.33±7.57a	115±2 9.3a	32±7.21a	57.72±15.7 2a	12.02±5.09a
K6	Control	91.67±6.81a	26.67±8.14a	119.3± 28.4a	30±6.08a	117.39±12. 97a	20.33±3.91a

	W3	91±6.08a	26.33±7.51a	119±18.5a	25.667±1.528a	95.67±3.25b	18.83±2.51a
	W5	86.33±2.31a	23±3.46a	113.3±22.5a	25±1.73a	85.45±3.75b	17.41±1.74a
	W7	86.333±1.155a	21.33±4.93a	103.7±20.8a	24±1.73a	79.61±6.92b	14.38±4.09a
K7	Control	108.67±3.21a	17±2.65a	84.67±14.47a	37.67±5.03a	81.43±5.25a	9.92±1.76a
	W3	105.67±4.93a	18.67±2.31a	99±11.53a	35.33±5.51a	76.47±2.77ab	10.22±1.036a
	W5	96.667±1.528b	19±3.61a	100.3±17.9a	36.33±5.13a	68.06±4.86bc	10.27±1.159a
	W7	93.333±1.528b	19.67±6.11a	94.3±29.6a	30±1a	62.74±4.58c	10.91±1.53a
K8	Control	100.33±1.53a	10±1.73a	65.33±8.33a	34±1a	62.5±3.65a	10.14±1.91a
	W3	97±2.65ab	9.667±1.528a	64.67±7.57a	31.667±1.528a	51.3±6.29ab	8.73±1.002ab
	W5	95.33±2.08ab	10±1a	61±6.56a	31.33±3.06a	45±5.81bc	7.423±0.35ab
	W7	93.667±1.528b	9.667±1.528a	55.33±9.71a	31±2.65a	37.33±3.94c	6.353±0.231b
K9	Control	108.33±8.33a	16±3a	79.33±11.93a	32.33±3.21a	71.5±5.15a	13.69±2.45a
	W3	105.33±5.51a	15.33±2.52a	77±11.36a	31±1a	60.77±4.92a	12.59±2.07a
	W5	103±2.65a	13.333±1.155a	64.67±5.77a	32±2.65a	56.65±9.57a	11.71±1.703a
	W7	104±2a	11±1a	60±6a	30±2a	51.28±14.11a	8.43±3.67a
K10	Control	101.67±2.89a	18.33±4.93a	84.7±25.5a	29.67±3.06a	93.6±27.5a	21.86±13.02a
	W3	104±1.73ab	18±5.29a	84±26.5a	34.333±1.155a	83.73±4.59a	18.06±8.13a
	W5	103.33±2.52ab	16.67±6.03a	81±28.5a	35.333±1.155ab	64.09±17.12a	14.85±5.58a
	W7	108.33±1.53b	16±7a	78.7±32.1a	36.33±2.31b	55.1±26.4a	11.88±8.31a
K11	Control	90.33±4.51a	22.67±5.51a	119.3±17.6a	35.67±3.21a	113.6±18.7a	21.63±3.37a

	W3	87.667±1.155a	23±5.2a	117±14.73a	33.67±3.21a	89.94±8.39ab	18.11±4.76ab
	W5	82.67±3.21ab	22.33±2.31a	112.33±8.96a	31.667±1.528a	74.39±7.5bc	15.64±4.23ab
	W7	78.67±3.21b	23±1a	107±4.58a	30±2a	52.06±8.84c	10.967±1.307b
ANOVA							
variety		*	*	*	*	*	*
Treatment		*	NS	NS	*	*	*
Variety*Treatment		NS	NS	NS	NS	NS	NS
Values are means ± standard division (SD) (n=3). Similar number in column are not significantly different at p>5%,* indicate significant differences at level 5%							

3.3 Efficacy of Water Deficit Stress on Total Phenolic and Total Flavonoids

The total Phenolic and total flavonoid contents are presented in table 5. The data indicate that the total phenolic content increased in K8 and K11 under stressed treatments, respectively. Furthermore, under W7, K8 showed a higher total phenolic content than K11. On the other hand, the total flavonoid content shows significant variation across the various treatments.

Table 5. Total phenolic and total flavonoid content

Sample		TPC **(µg/mL)**	TFC **(µg/mL)**
K8	Control	36.142±3.844d	3.1754±0.0799a
	W3	22.21±0.384e	3.0612±0.3013a
	W5	37.519±4.78cd	2.641±0.2356b
	W7	71.397±4.166a	2.2323±0.1482c
K11	Control	38.953±2.899cd	1.9997±0.0477cd
	W3	44.984±4.673bc	1.9532±0.084de
	W5	39.127±6.81cd	1.851±0.0494de
	W7	51.381±3.503b	1.7304±0.0569e

Values are means ± standard deviation (SD) (n=3). With 5% significant level

3.4 Antioxidant Activity

The responses of antioxidant activities are presented in Table 6. The values for all three assays (i.e., DPPH, ABTS, and reducing power) appear to be higher than the BHT values. The higher IC_{50} value denotes lower antioxidant activity. The IC_{50} values of DPPH, ABTS, and reducing power in K8 increased under a water-deficit condition. It was found that K8 treatment exhibited significantly lower IC50 values under water deficit than the control. In contrast, the IC_{50} values in K11 reduced when faced with water shortage. This means that the IC_{50} value in the control group was significantly higher than that in the treatment groups.

Table 6. Profile of antioxidant activities under water deficit

Sample		IC_{50} (µg/mL)		
		DPPH	ABTS	Reducing Power
K8	Control	12.117±2.046abc	1752.9±0.0091c	1237.7±0.0501cd
	W3	16.77±2.439a	1620.9±0.0068e	1199.1±0.002de
	W5	11.697±2.09abc	1365.8±0.0163f	1172.6±0.0034e
	W7	8.832±7.705c	1161.8±0.0163g	1168.2±0.0167e
K11	Control	10.123±0.976c	2007.4±0.0304a	1023±0.976f
	W3	10.689±0.177bc	1912.1±0.0054b	1260.9±0.0216c
	W5	11.045±0.61bc	1960.3±0.0449b	1363±0.0207b
	W7	16.261±2.781ab	1944.5±0.0548d	3721±0.0439a
BHT		6.406±0.78d	24.4±0.5h	21.1±1.4g

Values are means ± standard deviation (SD) (n=3). DPPH: 1, 1 diphenyl-2-picrylhydrazyl, ABTS: 2, 2, -azinobis (3-ethylbenzonline-6-sulfonic acid).

3.4.1 Correlation between Phenolic contents and Antioxidant Activities

There is a positive, robust, and significant correlation among total phenolic content, DPPH, and reducing power, as demonstrated in Table 7. More specifically, there exists a significant correlation between DPPH and reducing power at (0.001) p-value level. In contrast, there are no correlations between TFC, ABTS, and DPPH.

Table 7. Correlation value between total phenolic, flavonoid, and antioxidant activities.

Correlated compound	TPC	TFC	DPPH	ABTS
TFC	-0.023			
DPPH	0.653***	0.163		
ABTS	-0.145	0.225	-0.182	
Reducing power	0.768***	-0.198	0.018**	0.311

***, **: significant correlation at the level of 0.001 and 0.01, respectively. TPC: total phenolic content, TFC: total flavonoid content, DPPH: 1, 1 diphenyl-2-picrylhydrazyl, ABTS: 2, 2,-azinobis (3-ethylbenzonline-6-sulfonic acid) and reducing power.

3.4.2 Gas Chromatography-Mass Spectrometry (GC-MS)

The determination of volatile essential oil was acquired from the fractions of rice leaves by GC- MS, as summarized in table 9. The result indicates that essential compounds were detected in K8 more than K11. However, under water stress, most primary compounds disappeared, except for sucrose, which was detected.

Table 9. Basic compound recognition by GC.MS

	Sample	Major constituents	Retention time	Pick area intense of (1000)
K8	Control	Su, DoA, BeA, PhA, HeA	11.08, 15.85, 16.08, 22.09, 17.06	95.88, 241.1,3856.7,336.1,120
	W3	Su, PhA, Te-He, HeA, Th	11.08, 22.09, 15.84, 17.06, 6.12	95.88, 336.13, 4369, 120, 66.94
	W5	Su, Hy-Me, 2-Py, Hy, Pro, 4-HyA, 2-AzA, Hy	11.08, 5.44, 5.6, 6.25, 6.31, 16.73, 16.78, 19.23	95.88, 1210.2,2.15,24, 26.87, 17.21, 21.99, 17.27
	W7	Su, Hy-Me, Hy	11.08, 5.44, 19.23	95.88, 1210.2,17.27
K11	Control	Su, 2H-1Bn	11.08, 6.5	95.88, 39.45
	W3	Su, HeA, 3H-Py	11.08, 17.06, 6.13	95.88, 120, 325.62
	W5	Su, Oc, HeA, 1H-Te	11.08, 18.75, 17.06, 6.47	95.88, 398.84, 120, 54.76
	W7	Su	11.08	95.88

Abbreviations: GC-MS= gas chromatography mass spectrometry Su= Sucrose, DoA= Dodecanoic acid, 2-penten-1-yl ester, BeA= 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, PhA= Bis(2-ethylhexyl) phthalate-or-Phthalic acid, bis(2-ethylhexyl) ester, HeA= n-Hexadecanoic acid-OR-Hexadecanoic acid, Te-He= 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Th= Thymine, Hy-Me= Hydrazine, (1-methylethyl), 2-Py= 2-Pyrimidinamine, 4-methyl-6-phenyl, Hy= Hydrazinecarboxamide, Pro= Propanedinitrile, 2-[5-amino-4-cyano-2-methyl-2-(1-methylpropoxy)-3(2H)-furanyliden], 4-HyA= 4-Hydroxybutyric acid hydrazide, 2-AzA= (2-Aziridinylethyl)amine, Hydrazine, 2H-1Bn= 2H-1-Benzopyran-3-carbonitrile, 4-methyl-2-oxo-, 3H-Py= 3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl-, Oc= 9,12,15-Octadecatrienal, 1H-Te= 1H-Tetrazol-5-amine.

5. Discussion

The lack of water in rice production has been found to have a significant adverse effect on rice yields, which leads to extreme hunger, particularly in developing countries. For example, the total share of crop losses due to water deficit in Asia was $28 billion, while Africa accounted for $25 billion (FAO, 2015). While there are many types of rice varieties, different cultivars respond differently to the complex environmental conditions along with the molecular, biochemical and physiological reactions, which could have negative impacts on the growth and development of the plants (Meena et al., 2017).

In this study, we used leaf rolling, leaf drying, leaf withering, and leaf recovery as traits to evaluate the levels of tolerance and susceptibility of 11 rice varieties. Using the rice tolerance score recommended by IRRI, the K11 variety proved highly susceptible to water deficit. Contrastingly, K8 showed resistance to drought, as evidenced by leaf rolling, leaf drying, and leaf withering after 7 days of treatment. Furthermore, under persistent water deficit, K8 showed a higher recovery rate, while K11 showed the lowest level of leaf recovery. This result suggests that K11 is highly drought susceptible, and K8 is the most tolerant variety, as observed in the experiment. The response of rice plants under drought conditions can be visible in the retention of leaf withering, leaf rolling, and leaf drying and depicts the level of tolerance of the plant during growth (Hura et al., 2012). The study further examined the physical characteristics of the rice plant, considering height (cm), tiller number per hill, leaf number, root length (cm), root fresh weight (g) and dry root weight (g) under both drought and irrigated conditions. In the results, plant heights range from 77.33 to 115.67cm, while the tiller number per hill ranges from 10 to 26. The Leaf numbers span between 60 to 119, and the weight of the fresh is 37.33 to 117.39 (g), while the weight of the dry roots is between37.33 to 117.39 (g), and 7.423 to 20.33 (g). Farooq et al. (2009) found that increased vigor in rice plant parts, such as leaf area, leaf number, and tiller numbers per hill, supports this finding, and that higher drought scores are associated with increased vigor.

Interestingly, K8 shows the lowest numbers of plant heights and tillers per hill and K11 reveals the highest numbers of plant heights and tillers per hill, indicating that K8 is tolerant as K11 is susceptibility to drought. This result, therefore, implies that tiller number per hill, leaf number, fresh root weight, and root drought weight are significant determinants of the level of susceptibility and tolerance in rice. Generally, water deficit leads to oxidative stress and cell damage in the rice plant, resulting in the accumulation of phytochemicals such as total phenolic and total flavonoid contents and increased antioxidant activities (Nichols et al., 2015). Hence, we observed that the total phenolic content increased in K8 and K11 as water shortage intensified compared to the control group.

Some research points to the response of plants to drought and ultraviolet radiation and the involvement of sunshield in the build-up of phenolic acids, flavonoids, and antioxidant activities (Nichols et al., 2015). The data also show that with water shortages of up to 7 days, phenolic content in K8 rose dramatically compared to K11. In contrast, total flavonoid content shows significant

variation across treatments. Similarly, the total flavonoid content in K8 surpasses those of the other varieties and the control, with increasing treatment intensity.

A more fundamental benchmark for the quality and functionality of bioactive components in the food and pharmaceutical sectors is antioxidant activity, particularly DPPH and ABTS (Singh and Kumari, 2015). We juxtaposed the BHT standard with DPPH, ABTS, and reducing power, and the values for all three activities were lower than those for BHT, indicating higher antioxidant activity. The IC_{50} values for DPPH, ABTS, and reducing power in K8 increased significantly as water shortage progressed compared with the control group. In contrast, K11 showed lower IC50 values under water shortage, further demonstrating its sensitivity to drought conditions. The existence and amount of antioxidant capacity and phenolic acids are correlated with their activities (Sakthidevi and Mohan, 2013). The correlation between antioxidant activities and other phytochemicals was further determined during data analysis. Hence, a positive and significant correlation between DPPH and reducing power was observed (0.001) p-value level. However, there were no correlations between TFC, ABTS, and DPPH. \ An accurate analytical tool used in this study to identify the presence of phytochemicals, including volatile compounds in rice plant extract, is the Gas chromatography-mass spectrometry (GC-MS) (Tuyen et al., 2018). In the same vein, the quantification of volatile essential oil was achieved from the fraction of rice leaves by GC- MS. The analysis detected more basic compounds in K8 than K11. Surprisingly, when faced with a water shortage, the amount of the compound was undetected, except for sucrose. This result suggests that water stress significantly affects the presence of essential compounds in the rice plant, as demonstrated by this research.

6. Conclusion

The result of this study showed that drought stress increased total phenolic content in K8 and K11 varieties. K8 variety showed higher antioxidant activity under water-deficit conditions; however, K11 showed lower antioxidant activity and reducing power. This result suggests that water stress remarkably affects the presence of essential compounds in the rice plant. It was observed that K8 variety of rice was the most tolerant, whereas K11 the most susceptible to drought stress.

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